



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A01N 43/60	A1	(11) International Publication Number: WO 99/37152 (43) International Publication Date: 29 July 1999 (29.07.99)
<p>(21) International Application Number: PCT/GB99/00275</p> <p>(22) International Filing Date: 27 January 1999 (27.01.99)</p> <p>(30) Priority Data: 9801712.2 27 January 1998 (27.01.98) GB</p> <p>(71) Applicant (for all designated States except US): ROTHAMSTED EXPERIMENTAL STATION [GB/GB]; Harpenden, Rothamsted, Hertfordshire AL5 2JQ (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): PETTERSSON, Jan [SE/SE]; Swedish University of Agricultural Sciences, Dept. of Entomology, P.O. Box 7044, S-750 07 Uppsala (SE). BIRKETT, Michael, Alexander [GB/GB]; Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ (GB). PICKETT, John, Anthony [GB/GB]; Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ (GB).</p> <p>(74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: PYRAZINES AS ATTRACTANTS FOR INSECTS OF ORDER COLEOPTERA</p> <p>(57) Abstract</p> <p>Pyrazine derivatives of general formula (I) can be used as an attractant for conspecific adults of the family Coccinellidae belonging to the order Coleoptera to assist in pest control, particularly control of pests of the family Aphididae.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

PYRAZINES AS ATTRACTANTS FOR INSECTS OF ORDER COLEOPTERA

- 5 The present invention relates to the control of harmful agricultural and horticultural insect pests, principally of the order Homoptera specifically relating to the family Aphididae, through the use of signal substances (semiochemicals) that alter the behaviour of populations of predators, belonging to the order Coleoptera specifically relating to the family Coccinellidae, and which are predator-derived.
- 10 In the control of pests, especially insects, of plants of agricultural and horticultural importance, the aim is to subject pests to a pesticide through contact or ingestion in such a way that there is optimal exposure of the insect to the pesticide, and minimal contact of the pesticide with the plants to be protected and the soil in which they grow. In integrated pest management (IPM) programmes for the control of plant
- 15 pests, the aim is to incorporate the use of pesticides with other methods of pest control, such that the harmful effects of pesticides on warm-blooded animals, beneficial insects and the surrounding environment are minimised. One way is through the use of signal substances (semiochemicals) that influence the behaviour or development of insects, *via* signalling and not *via* direct physiological action. The
- 20 most active semiochemicals are pheromones, which are semiochemicals acting only between members of the same species. For pests, semiochemicals can be used either for the disruption of the pairing process, for monitoring of pest populations prior to pesticide usage, or to lead pests to a source of pesticide or bio-pesticide that is provided in discrete regions in the crop-area. Semiochemicals can also be used to
- 25 draw pest predators and parasites into the vicinity of the area of vegetation to be protected. Effective control of insect pests using semiochemicals requires slow release of the substances from a matrix, substrate or microcapsule, such that the substances are protected from the weather and light, and are released in biologically effective amounts over a prolonged period of time. Pheromones can be sexual signal
- 30 or aggregation signal substances, where the former is produced in most cases by the

adult females to attract individuals of the opposite sex, and in the case of the latter are produced by one or other of the sexes, to which both sexes are attracted. Pheromones are often volatile and their effect on attraction can reach over long distances. Kairomones are semiochemicals of advantage to the recipient but
5 disadvantage the emitter. For the predators of pests they are produced by the prey insects.

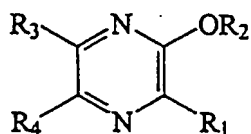
Insects of the order Homoptera representative of the family Aphididae ("aphids" *e.g.* greenfly and blackfly) are major agricultural and horticultural pests in Western
10 and Northern Europe (Pickett *et al.*, *Ann. Rev. Entom.*, 37, 67-90 (1992)). Predators and parasites of pest aphids are beneficial because of the role they have in controlling prey populations, and could be exploited more effectively as biological control agents by use of semiochemicals. In relation to the aims of the invention, very little is known of the identity of predator-derived signal substances that
15 influence the behaviour of aphid predators belonging to the family Coccinellidae ("ladybirds", "ladybeetles", "ladybugs"). Previous reports on aphid-derived substances *i.e.* kairomones are limited, for example it has been shown that populations of the aphidophagous ladybird *Hippodamia convergens* Guerin were affected by food sprays containing artificial aphid honeydew, but this was later
20 shown to be only an "arrestant" response and not "attraction" (Hagen *et al.*, *Proc. Tall Timbers Conf. On Ecol, Animal Control and Habitat Management*, Vol. 2, 59-81 (1971)). It is, though, well documented that ladybirds aggregate in the autumn prior to overwintering (M.E.N. Majerus, in *Ladybirds* (1994)). However, no compounds causing attraction of ladybirds or increased foraging of ladybirds thereby
25 increasing predation have been reported in the scientific literature.

Ladybirds and many other insect species have been shown to possess chemical means for defence. For example, the widespread occurrence of methylpyrazines as communal insect defence odours was reported by Moore *et al* (*Chemoecology* 1 43-
30 51 (1990)). This paper also reported the tentative identification of 2-isopropyl-3-

methoxy pyrazine, 2-*sec*-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine as warning chemicals which were components of a number of aposematic insects and their host plants. The compounds were identified using selected ion monitoring of volatile extracts and comparison of gas chromatography (GC) retention times. These compounds were also tentatively identified as components of the aposematic beetle *Metriorrhynchus rhipidus* (Moore, B. P., & Brown, W. V., *Insect Biochem* 11 493-499 (1981)). Both of these papers only describe the biological roles of these pyrazines as either alerting signals or warning odours utilised by aposematic insects to repel would-be predators.

A pheromonal cue for ladybirds has now been discovered which can be used to preserve overwintering populations for later use in the control of aphid pests. Surprisingly, it has been found that the methoxypyrazine compounds identified as warning compounds or alerting odours also have a role as ladybird attractants.

According to a first aspect of the invention there is provided the use of a compound of general formula (I) as an attractant for conspecific adults of the family Coccinellidae belonging to the order Coleoptera



(I)

in which:

R¹ is H or (C₁-C₄)alkyl

R² is CH₃ or C₂H₅

R³ is H

R⁴ is H

As used herein the term "(C₁-C₄)alkyl" refers to a straight or branched chain hydrocarbon molecule having from one to four carbon atoms. Illustrative of such groups are methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, *sec*-butyl or *tert*-butyl.

5 The discovery of the invention means that the amount of insecticides, which are often toxic to warm-blooded animals and useful insects, required for the treatment of herbaceous areas can be restricted considerably. This novel procedure which is also advantageous from the ecological point of view is also applicable to those concepts that are based on integrated plant protection and have already received considerable
10 attention. The invention is applicable to a wide range of integrated pest management (IPM) schemes in use for protection of agricultural and horticultural systems.

Preferred compounds according to general formula (I) are 2-isopropyl-3-methoxy pyrazine, 2-*sec*-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine.

15

The family Coccinellidae includes the following species: *Coccinella septempunctata* L., *Adalia bipunctata* L., *Hippodamia convergens* Guerin, *Epilachna cucurbitae* Richards, *Epilachna 26-punctata* (Boisd.), *Subcoccinella 24-punctata* L., *Rhyzobius ventralis* Erichson, *Rodalia cardinalis* Mulsant, *Rodatus boucardi* Crotch,
20 *Cryptolaemus montrouzierii* Mulsant, *Paraprius australasiae* (Boisd.), *Micraspis frenta* (Erichson), *Illeus* sp., *C. transversalis* and *Harmonia conformis* (Boisd.). In a preferred embodiment of the present invention, the attractant compound of general formula (I) may be used to attract conspecific adults of the species *Coccinella septempunctata*.

25

According to a second aspect of the present invention there is provided the use of a compound according to general formula (I) in the preparation of an agent for attracting conspecific adults of the family Coccinellidae belonging to the order Coleoptera. The compounds of general formula (I) can be formulated in any
30 appropriate carrier medium

Formulations of a compound of general formula (I) may include aqueous and non-aqueous solutions which may optionally contain anti-oxidants, buffers, bacteriostats and suspending agents or thickening agents. Excipients which may be used include
5 water, alcohols, polyols, glycerine and vegetable oils, for example. Organic solvents in addition to alcohols and polyols may also be used in preparing formulations where acceptable. The compositions may be stored in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the liquid
10 carried, for example water, immediately prior to use. The formulation may also be activated by the action of moisture from the environment in the form of condensation or humidity, rain etc. Extemporaneous solutions and suspensions may be prepared from powders, granules and tablets. The preparations may also be formulated under sterile conditions, if necessary. The formulations may contain preserving agents,
15 solubilising agents, stabilising agents, wetting agents, emulsifiers, colourants, odourants, salts (molecules of the present invention may themselves be provided in the form of a biochemically active salt), buffers, coating agents or antioxidants. They may also contain bioactive agents in addition to a compound according to general formula (I) of the present invention.

20 It is also envisaged that formulations may be prepared by incorporating a compound of general formula (I) as previously defined into a solid or a gelatinous base for release of the chemoattractant. Solid bases include but are not limited to natural or artificial polymer materials, suitably in the form of sheets or membranes
25 incorporating the chemoattractant, or alternatively comprising a number of woven fibres of material coated with the chemoattractant or otherwise containing the chemoattractant within its structure. Potential solid materials include, cellulose, nitrocellulose in the form of membranes or woven fibrous materials. Fibrous materials may be manufactured from any convenient material, including, cotton,
30 wool, silk, or a polymer, or a combined multi-component fibre. Where the thread

has a polymer component, the polymer may be cellulose, polyethylene (suitably, ultra-high modulus polyethylene) polyester, terylene, nylon, lyocell (Tencel™), or any other suitable polymer fibre. Suitably the article can be composed of fibres of different degrees of microfibrillation. The chemoattractant may also be incorporated
5 within a gelatinous material or a gel composed of natural or synthetic materials, such as for example resins, oils or waxes.

According to a third aspect of the present invention there is provided a sustained release formulation comprising a compound of general formula (I) for use as a
10 chemoattractant for conspecific adults of insects belonging to the family Coccinellidae of the order Coleoptera. Sustained release formulations may be prepared by any appropriate means for the release of volatile organic compounds, including formulations described above in relation to the previous aspect of the invention. Such compositions may be presented as discrete units such as capsules,
15 inserts, membranes, woven fibres or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as foams or whips; or as emulsions); as solids or gels incorporating the chemoattractant, including membranes, woven fibres and other substrates. Suitable excipients for tablets, inserts or capsules include lactose, maize starch or derivatives thereof, stearic acid
20 or salts thereof. Alternative excipients can include, for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. Prior to use the sustained release formulation may be appropriately packaged to prevent release until desired. The sustained release formulation may though be activated by environmental conditions, such as for example moisture. Where the formulation is prepared as a capsule, the
25 capsule may be formulated from any suitable porous material including hard or soft gelatin.

According to a fourth aspect of the present invention there is provided a method for controlling an insect pest species comprising administering a formulation as
30 previously described to an infested area wherein the formulation attracts conspecific

adults of a predator non-pest insect of the family Coccinellidae belonging to the family Coleoptera.

5 Pest species which may be controlled in accordance with this aspect of the invention include the natural prey species of the Coccinellid insect to be attracted. In general , it is to insect pests of the order Homoptera, specifically relating to the family Aphididae. Where the predator insect is *Coccinella septempunctata* L. an appropriate pest species may be an aphid species also known as "green fly" or "black fly".

10

According to a fifth aspect of the present invention there is provided a kit comprising a sustained release formulation as previously defined and a device for containing conspecific adults of the family Coccinillidae belonging to the order Coleoptera. Alternatively, the kit may comprise a compound of general formula (I) which is not
15 prepared as a sustained release formulation, optionally together with a carrier. The kits in accordance with this aspect of the invention may be designed to any appropriate dimensions to be used as containers for Coccinellid insects which have been attracted into the devices. The devices can then be used as environmental pest control means when placed at a site of pest infestation or to prevent infestation in an unaffected site.
20 Typically, these devices will be used in greenhouses or commercial glasshouses, or any other construction to cover or otherwise enclose a plant growing area. These devices may also find application in the open where particular pest infestation problems are encountered. As the chemoattractant formulation is dissipated in use, it may be replaced in the device by another chemoattractant
25 formulation, suitably in the form of an insert. Alternatively, the device may be re-dosed with the formulation.

30

Preferred aspects for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The invention will now be further described by way of example only with reference to the following Examples and Drawings which are included for the purposes of illustration only and are not to be construed as being limiting on the present invention. Reference is also made to a number of figures in which:

5

FIGURE 1 shows a gas chromatogram (50m x 0.32mm i.d. HP-1 column) of vacuum distillate from extract of *C. septempunctata* adults; the arrow indicates the peak at which organoleptic activity was detected.

10

FIGURE 2 shows a mass spectrum from the peak marked by an arrow in Figure 1. Coupled GC-MS was provided by a capillary GC column (50m x 0.32mm i.d. HP-1) fitted with an on-column injector coupled directly to a mass spectrometer (VG Autospec, Fisons Instruments).

15

Organoleptic evaluation of volatile compounds isolated from *C. septempunctata*

Organoleptic evaluation of separated components in GC effluent is an effective technique, though relatively underexploited in chemical ecology, for the identification of volatiles responsible for the characteristic odour of extracts collected from biological systems (*e.g.* Zeng *et al.*, *J. Chem. Ecol.*, 1991). In a similar manner to that of coupled GC-electrophysiology (L.J. Wadhams, in *Chromatography and Isolation of Insect Hormones and Pheromones*, 1990), where GC-separated components are passed over an insect antenna, the volatiles are presented to the human nose for the location of distinctive odours. Although there are similarities in olfactory responses by human beings and arthropods including insects, when the organoleptic technique is applied to the identification of insect semiochemicals, extreme caution must be exercised in ensuring that compounds detected by human olfaction are of significance to the insects under investigation. When heteroatoms are incorporated, human olfaction, on a body weight basis, can show similar sensitivity to that of insects (Seifert, *J. Agric. Food Chem.*, 18, (2), 246-249 (1970)).

20
25
30

The seven-spot ladybird *Coccinella septempunctata* L. is one of the most important aphidophagous species. The behavioural responses of *C. septempunctata* adults to conspecifics and to an extract of isolated *C. septempunctata* volatiles in a modified
5 Pettersson olfactometer for use with walking insects were monitored (J. Pettersson, *Entomol. Scand*, 1, 63, (1970)). Tests with male and female *C. septempunctata* showed significant attraction to volatiles released by conspecific adults. The vacuum distillate isolated from an extract (chloroform) of *C. septempunctata* adults was also attractive. Human olfactory assessment of the whole extract revealed that the
10 characteristic odour of ladybirds had been retained. Organoleptic evaluation of the sample separated on a non-polar HP-1 column suggested that a single area of interest was responsible for this characteristic odour. This compound was tentatively identified from coupled GC-MS as 2-isopropyl-methoxypyrazine (NIST, 1990) and this was confirmed by peak enhancement with authentic compound, obtained from
15 the Aldrich Chemical Company (Gillingham, UK). Olfactometer tests with adult *C. septempunctata* indicated that this pyrazine accounted for the activity of the extract. The behavioural response appeared to take on the form of an "attraction-arrestant" response, whereby the beetles were initially attracted to the volatile component source and thereafter exhibited reduced mobility.

20

Example 1: Pheromone Isolation Example

Adult male and female *C. septempunctata* (ca. 1000) were collected from grounds surrounding IACR-Rothamsted and kept in ventilated sandwich boxes at 20°C until required, were cooled with liquid nitrogen and extracted with freshly distilled
25 chloroform (2 x 200 ml) for 24h (48h in total) at 25°C. The combined extracts were dried using anhydrous magnesium sulphate, filtered, and evaporated to ca. 5 ml. Volatiles were collected by vacuum distillation under reduced pressure (21h, 0.03 torr) and the resulting extract concentrated under a stream of nitrogen to 100 µl and stored in a tightly capped microvial at -20°C.

30

Example 2: Pheromone identification Example

To determine which of the volatile components resembled, to human beings, the characteristic odour of ladybirds, a panel of two judges (MAB, JAP) was assembled to perform organoleptic evaluation. Prior to chromatographic separation, the odour of the entire vacuum distillate was assessed. Olfactory sampling of the GC effluent began 2 minutes after injection. Volatiles were separated on an AI93 GC equipped with a cold on-column injector, a flame ionization detector (FID) and a 50 m x 0.32 mm i.d. HP-1 capillary column. The oven temperature was maintained at 40°C for 2 minutes and programme at 5°/minute to 100°C, then a 10°/minute to 250°C. The carrier gas was hydrogen.

Coupled GC-MS was provided by a capillary GC column (50 m x 0.32 i.d. HP-1) fitted with an on-column injector directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments). Ionization was by electron impact at 70eV, 250°C. The oven temperature was maintained at 30°C for 5 minutes and then programmed at 50/minute to 250°C. Tentative identification by GC-MS was confirmed by peak enhancement on GC with authentic 2-isopropyl-3-methoxypyrazine (97%) (Aldrich Chemical Company, Gillingham, UK).

Example 3: Behavioural bioassay example

The olfactometer has already been described in principle in the literature (J. Pettersson, *Entomol. Scand.* 1, 63 (1970)), and is modified for use with walking insects. This comprises a weak airstream being directed towards the centre of the olfactometer from two drawn-out arms to which volatile sources are applied at the inlets. The arena is divided into two zones with a neutral zone in the centre. The olfactometer is surrounded by white paper screen (30 cm high) to minimise the effect of visual stimuli in the environment. Both arms are supplied with moist filter paper (2 x 2 cm) to minimize differences in relative humidity. To avoid arena contamination the bottom of the olfactometer is covered with white paper which is changed between experiments.

For each experiment, one *C. septempunctata* adult was introduced into the centre of the chamber and its position noted every 2 minutes for 20 minutes. Each experiment was replicated 5 or 10 times and the results analysed by *t*-test; the number of visits into the treatment arm was compared with visits into the treatment arm was compared with visits to the control arm. Stimuli comprised: (1) one *C. septempunctata* adult, either male or female; (2) *C. septempunctata* vacuum distillate (0.04 insect equivalents containing 0.05 ng/ μ l 2-isopropyl-3-methoxypyrazine); (3) 2-isopropyl-3-methoxypyrazine at 0.05 ng/ μ l. Treatments (2) and (3) were applied in 0.5 μ microcaps. If the insect did not move between two observations, the experiment was terminated and the data discarded. Results of these studies are shown in Table 1.

Table 1

Responses of overwintering *Coccinella septempunctata* adults in the two-arm olfactometer*

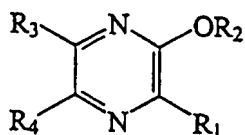
Stimulus	Mean no. Observations in Treated arm	Mean no. observations in control arm**	<i>P</i>	Replicates (N)
<i>C. septempunctata</i> (single adult)	5.8 (1.32)	2.9 (1.45)	<0.05	10
<i>C. septempunctata</i> (vacuum distillate)	6.8 (1.92)	3.2 (1.92)	<0.05	5
2-isopropyl-3-methoxypyrazine	8.4 (1.90)	1.6 (1.90)	<0.01	10

*Cumulative counts over 20 minutes. Figures in brackets refer to standard deviation.

** Control = solvent (diethyl ether)

CLAIMS

1. The use of a compound of general formula (I) as an attractant for conspecific adults of the family Coccinellidae belonging to the order Coleoptera



(I)

in which:

R¹ is H or (C₁-C₄)alkyl

R² is CH₃ or C₂H₅

R³ is H

R⁴ is H

2. A use as claimed in claim 1, in which the compound is 2-isopropyl-3-methoxy pyrazine, 2-*sec*-butyl-3-methoxypyrazine or 2-isobutyl-3-methoxypyrazine.

3. A use as claimed in claim 1 or claim 2, in which the insect of the family Coccinellidae is *Coccinella septempunctata* L..

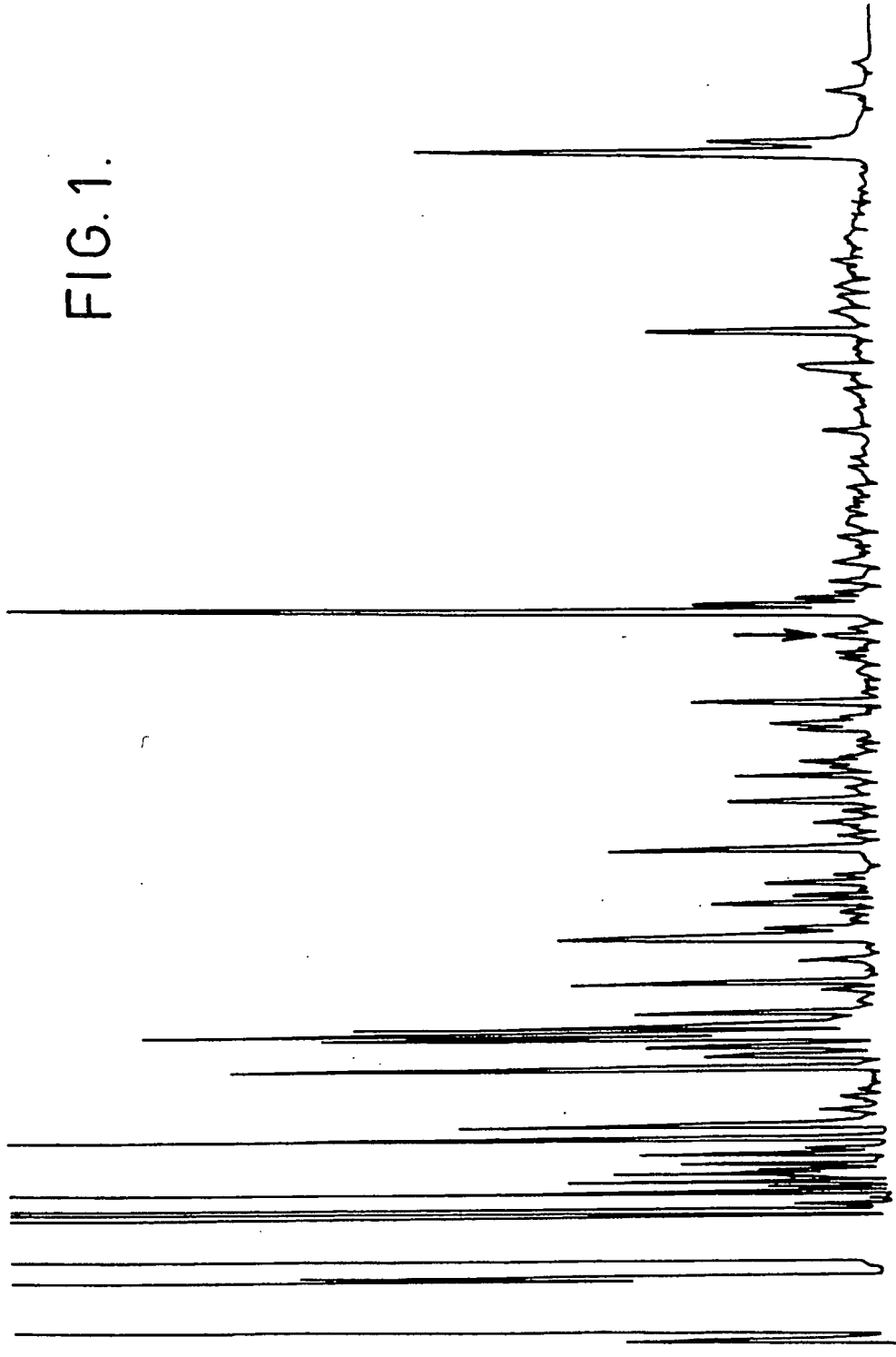
4. The use of a compound according to general formula (I) in the preparation of an agent for attracting conspecific adults of the family Coccinellidae belonging to the order Coleoptera.

5. A sustained release formulation comprising a compound of general formula (I) for use as a chemoattractant for conspecific adults of insects belonging to the family Coccinellidae of the order Coleoptera.

6. A method for controlling an insect pest species comprising administering an agent as defined in claim 4 or a formulation as defined in claim 5 to an infested area wherein the formulation attracts conspecific adults of a predator non-pest insect of the family Coccinellidae belonging to the family Coleoptera.
7. A method as claimed in claim 6, in which the pest species is of the family Aphididae.
8. A kit comprising a sustained release formulation as defined in claim 5 and a device for containing conspecific adults of the family Coccinellidae belonging to the order Coleoptera.

1 / 2

FIG. 1.

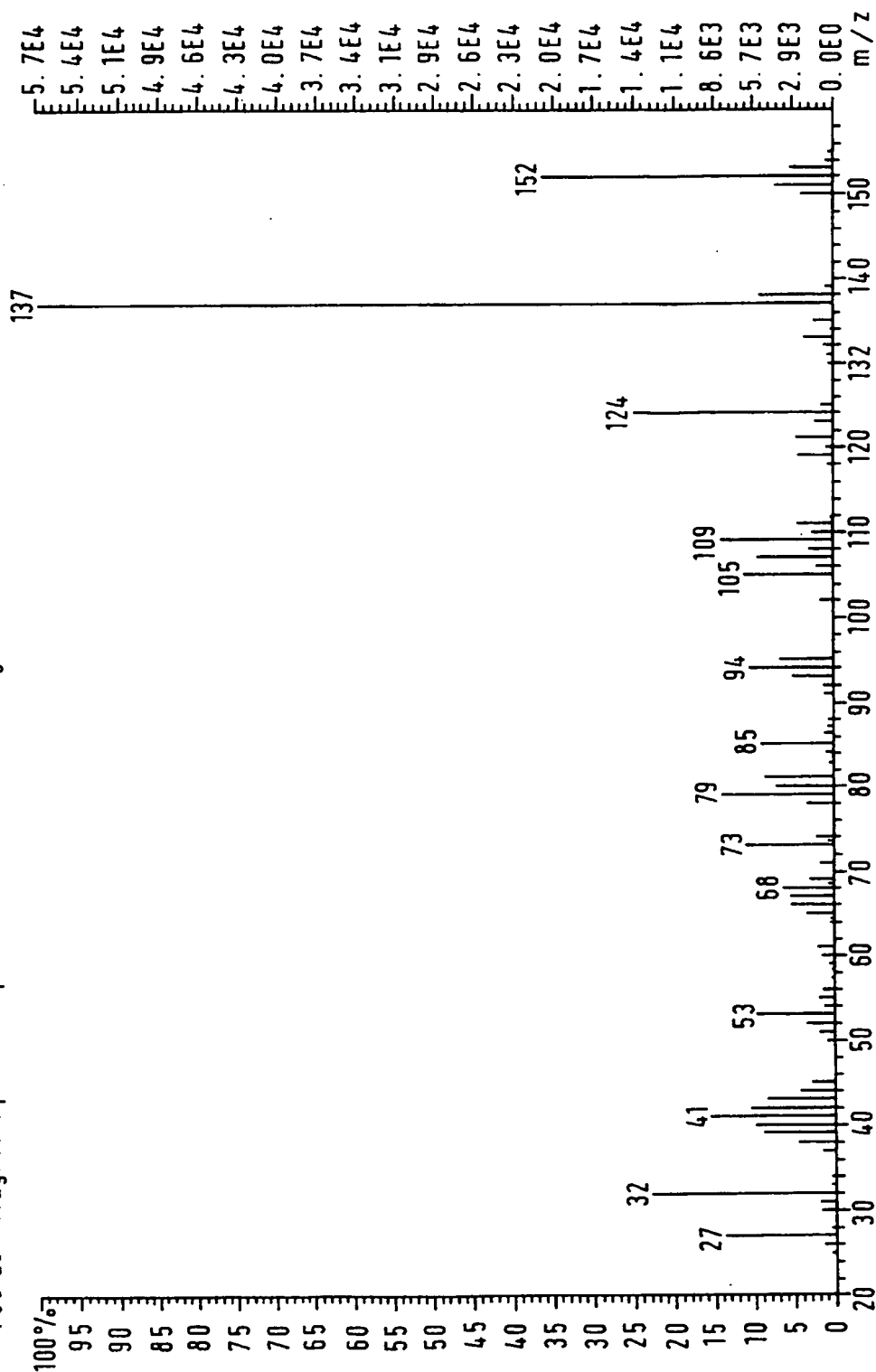


SUBSTITUTE SHEET (RULE 26)

2 / 2

FIG. 2.

File: P11M96C Ident: 849-858 Win 1000PPM Acq: 26-FEB-1997 09:56:48 Cal: 25FEBPFK_1
70S EI + Magnet BpM: 137 BpI: 57174 TIC: 313290 Flags: HALL



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/00275

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N43/60

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CHEMICAL ABSTRACTS, vol. 129, no. 12, 21 September 1998 Columbus, Ohio, US; abstract no. 146971, S. AL ABASSI ET AL: "Ladybird beetle odor identified and found to be responsible for attraction between adults" XP002103470 see abstract & CELL. MOL. LIFE SCI., vol. 54, no. 8, 1998, pages 876-879, --- -/--	1-8



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 May 1999

Date of mailing of the international search report

07/06/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lamers, W

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/00275

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BIOLOGICAL ABSTRACTS, vol. 63, Philadelphia, PA, US; abstract no. 7466, A.A.B. SAAD ET AL.: "ATTRACTION OF INSECT TO POTATO PLANTS THROUGH USE OF ARTIFICIAL HONEYDEWS AND APHID JUICE" XP002103467 see abstract & ENTOMOPHAGA, vol. 21, no. 1, 1976, pages 49-57, ---</p>	1-8
A	<p>BIOLOGICAL ABSTRACTS, vol. 1994, Philadelphia, PA, US; abstract no. 344099, C.SENGOCA ET AL.: "Responses of the different instar predator, Coccinella septempunctata L. (Coleoptera: Coccinellidae) to the kairomones produced by the prey and non-prey insects as well as the predator itself" XP002103468 see abstract & ZEITSCHRIFT FÜR PFLANZENKRANKHEITEN UND PFLANZENSCHUTZ, vol. 101, no. 2, 1994, pages 173-177, ---</p>	1-8
A	<p>BIOLOGICAL ABSTRACTS, vol. BR27, Philadelphia, PA, US; abstract no. 27972, K.NAKAMUTA: "APHID BODY FLUID STIMULATES FEEDING OF A PREDATORY LADY BEETLE COCCINELLA-SEPTEMPUNCTATA COLEOPTERA COCCINELLIDAE." XP002103469 see abstract & APPL. ENTOMOL. ZOOLOG., vol. 19, no. 1, 1984, pages 123-124, ---</p>	1-8
A	<p>CHEMICAL ABSTRACTS, vol. 115, no. 7, 19 August 1991 Columbus, Ohio, US; abstract no. 68762, P.B.MOORE ET AL.: "Methylalkylpyrazines in aposematic insects, their hostplants and mimics" XP002103471 cited in the application see abstract & DATABASE CABA STN-International STN-accession no. 91:65212, see abstract & CHEMOECOLOGY, vol. 1, no. 2, 1990, pages 43-51, -----</p>	1-8